

WO 97/40156

PCT/US97/06470

63
-68/1-

1. A mutant *ras* peptide comprising a peptide or analog thereof which elicits mutant *ras*-p21 protein or peptide specific cytotoxic T lymphocytes, wherein the amino acid at position 12 is selected from the group consisting of aspartic acid, valine, cysteine, alanine, arginine and serine and wherein the peptide has at least one amino acid substitution at a position distinct from position 12, said substitution provides an enhanced T-cell response in comparison to the T-cell response of the non-substituted peptide.
2. The mutant *ras* peptide according to claim 1, wherein the amino acid at position 12 is aspartic acid, valine or cysteine.
3. The peptide according to claim 1, wherein the substitution is at position 5, position 7 or position 5 and position 7.
4. The peptide according to claim 1, 2 or 3 wherein the substitution is a tyrosine in place of a lysine at position 5.
5. A mutant *ras* peptide according to claims 1-3 or 4, wherein the peptide contains both CD4-positive and CD8-positive T cell epitopes in an overlapping configuration.
6. The peptide according to claim 5, wherein the peptide comprises an amino acid sequence of about 8 to about 13 amino acids.
7. The peptide according to claim 5, wherein the amino acid residue at position 12 is selected from the group consisting of aspartic acid, valine, cysteine, alanine, arginine, and serine.
8. The peptide according to claim 7, wherein the amino acid at position 12 is aspartic acid, valine or cysteine.
9. A mutant *ras* peptide comprising an amino acid sequence SEQ ID NO:11.

WO 97/40156

PCT/US97/06470

64
-6971-

10. A mutant *ras* peptide comprising
Xaa₁ Leu Xaa₂ Val Val Gly Ala Xaa₃ Gly Val;
wherein Xaa₁ is the amino acid lysine or tyrosine;
wherein Xaa₂ is an amino acid;
wherein Xaa₃ is selected from the group consisting of aspartic acid, valine,
cysteine, alanine, arginine, and serine;
with the proviso that when Xaa₁ is valine, Xaa₃ is tyrosine
and said peptide elicits peptide-specific human CD8+ cytotoxic T
lymphocytes.

11. The mutant *ras* peptide according to claim 10 wherein the peptide
comprises an amino acid sequence of about 13 amino acids.

12. The mutant *ras* peptide according to claim 10 wherein the peptide
comprises an amino acid sequence of about 10 amino acids.

13. The mutant *ras* peptide according to claim 10, 11 or 12 wherein
Xaa₁ is tyrosine.

14. The mutant *ras* peptide according to claim 10, 11, 12 or 13 wherein
Xaa₂ is selected from the group consisting of valine, tryptophan, leucine, tyrosine,
and phenylalanine.

15. The mutant *ras* peptide according to claim 10, 11, 12, 13 or 14
wherein Xaa₁ is tyrosine and Xaa₃ is aspartic acid.

16. The mutant *ras* peptide according to claim 10, 11, 12, 13 or 14
wherein Xaa₂ is tyrosine and Xaa₁ is valine.

17. The mutant *ras* peptide according to claim 10, 11, 12, 13 or 14
wherein Xaa₁ is tyrosine and Xaa₃ is cysteine.

18. The mutant *ras* peptide according to claim 10, 11, 12, or 14 wherein
Xaa₁ is lysine and Xaa₃ is aspartic acid.

19. The mutant *ras* peptide according to claim 10, 11, 12 or 14 wherein
Xaa₁ is lysine and Xaa₃ is valine.

WO 97/40156

PCT/US97/06470

65
-70/1-

20. The mutant *ras* peptide according to claim 10, 11, 12 or 14 wherein Xaa₁ is lysine and Xaa₂ is cysteine.

21. The mutant *ras* peptide according to claim 10, 11, 12, 18 or 19 wherein Xaa₁ is lysine and Xaa₂ is tryptophane.

22. The mutant *ras* peptide according to claim 10, 11, 12, 15, 16 or 17 wherein Xaa₁ is tyrosine and Xaa₂ is tryptophan.

23. The mutant *ras* peptide according to claim 10, 11 or 12 wherein Xaa₁ is lysine, Xaa₂ is tryptophan, and Xaa₃ is selected from the group consisting of aspartic acid, valine and cysteine.

24. The mutant *ras* peptide according to claim 10, 11 or 12 wherein Xaa₁ is tyrosine, Xaa₂ is tryptophan and Xaa₃ is selected from the group consisting of aspartic acid, valine and cysteine.

25. A mutant *ras* peptide-carrier molecule conjugate comprising the mutant *ras* peptide according to claims 1-23 or 24 and a carrier molecule, said carrier molecule enhances the immunogenicity of the peptide.

26. The mutant *ras* peptide-carrier molecule conjugate according to claim 25 wherein the carrier molecule is selected from the group consisting of influenza peptide, tetanus toxoid, tetanus toxoid-CD4 epitope, *Pseudomonas* exotoxin A and poly-L-lysine.

27. An immunogen for eliciting mutant *ras* peptide-specific human CD8⁺ cytotoxic T lymphocytes comprising a mutant *ras* peptide according to claims 1-23 or 24 or combination thereof, said immunogen elicits mutant *ras* peptide-specific human CD8⁺ cytotoxic T lymphocytes.

WO 97/40156

PCT/US97/06470

-7171 -
66

28. An immunogen comprising a mutant *ras* peptide wherein the mutant *ras* peptide is generated from a 13 mer mutant *ras* peptide, said 13 mer mutant *ras* peptide comprising:

Xaa₁ Leu Xaa₂ Val Val Gly Ala Xaa₃ Gly Val Gly Lys Ser
wherein Xaa₁ is the amino acid lysine or tyrosine;
wherein Xaa₂ is an amino acid;
wherein Xaa₃ is selected from the group consisting of aspartic acid, valine, cysteine, alanine, arginine, and serine;
with the proviso that when Xaa₂ is valine, Xaa₃ is tyrosine;
said immunogen elicits mutant *ras* peptide-specific human CD8⁺ cytotoxic T lymphocytes.

29. An immunogen for eliciting mutant *ras* peptide-specific human CD8⁺ cytotoxic T lymphocytes comprising a mutant *ras* peptide consisting essentially of:

Tyr Lys Leu Val Val Gly Ala Xaa
wherein Xaa is selected from the group consisting of aspartic acid, valine, cysteine, alanine, arginine, and serine; and said peptide elicits peptide-specific human CD8⁺ cytotoxic T lymphocytes.

30. The immunogen according to claim 29 wherein the human CD8⁺ cytotoxic T lymphocytes are HLA-A2 restricted.

31. The immunogen according to claim 29 or 30 wherein the mutant *ras* peptide consists of SEQ. ID NO.: 6.

32. A pharmaceutical composition comprising the mutant *ras* peptide of claims 1-30 or 31 and a pharmaceutically acceptable carrier.

33. The pharmaceutical composition according to claim 32, further comprising a biological response modifier.

34. The pharmaceutical composition according to claims 32 or 33, further comprising an adjuvant, a liposome formulation, or an antigen presenting cell.

W.M.G.7

WO 97/40156

PCT/US97/06470

67
-721-

35. The pharmaceutical composition according to claim 34 wherein the adjuvant is RIBI Detox™, QS21, alum or incomplete Freund's adjuvant.

36. The pharmaceutical composition according to claims 33, 34 or 35 wherein the biological response modifier is selected from the group consisting of interleukin 2.

37. The pharmaceutical composition according to claims 32-35 or 36, further comprising interleukin 2, interleukin 6, interleukin 12, interferon, tumor necrosis factor, GM-CSF, cyclophosphamide, β -microglobulin or combinations thereof.

38. A pharmaceutical composition comprising a combination of at least two mutant *ras* peptides and a pharmaceutically acceptable carrier, said combination of mutant *ras* peptides selected from the group consisting of:

A) Xaa₁ Leu Xaa₂ Val Val Gly Ala Xaa₃ Gly Val;

wherein Xaa₁ is the amino acid lysine or tyrosine;

wherein Xaa₂ is an amino acid;

wherein Xaa₃ is selected from the group consisting of aspartic acid, valine, cysteine, alanine, arginine, and serine;

with the proviso that when Xaa₂ is valine, Xaa₁ is tyrosine,

and said peptide elicits peptide-specific human CD8⁺ cytotoxic T

lymphocytes;

B) Tyr Lys Leu Val Val Val Gly Ala Xaa

wherein Xaa is selected from the group consisting of aspartic acid, valine, cysteine, alanine, arginine, and serine;

and said peptide elicits peptide-specific human CD8⁺ cytotoxic T

lymphocytes; and

C) Xaa₁ Leu Xaa₂ Val Val Gly Ala Xaa₃ Gly Val Gly Lys Ser

wherein Xaa₁ is the amino acid lysine or tyrosine;

wherein Xaa₂ is an amino acid;

WO 97/40156

PCT/US97/06470

68

-731 -

wherein Xaa, is selected from the group consisting of aspartic acid, valine, cysteine, alanine, arginine, and serine;

with the proviso that when Xaa₂ is valine, Xaa₁ is tyrosine and said peptide elicits peptide-specific human CD8⁺ cytotoxic T lymphocytes; and

D) combinations of A, B and C.

39. A mutant *ras* peptide-pulsed antigen presenting cell, said cell pulsed with the mutant *ras* peptide according to any of claims 1-24, or 27-31.

40. A pharmaceutical composition comprising a mutant *ras* peptide-pulsed antigen presenting cell according to claim 39 and a pharmaceutically acceptable carrier.

41. The pharmaceutical composition according to claims 39 or 40, wherein the antigen presenting cell is a dendritic cell, a B lymphocyte, a monocyte or a macrophage.

42. An isolated DNA sequence comprising a DNA sequence thereof encoding a mutant *ras* peptide according to claims 1-24 or 27-31.

43. A plasmid or virus vector comprising the mutant *ras* peptide DNA sequence of claim 42.

44. A vector according to claim 43 wherein the vector is *E. coli* plasmid, a Listeria vector, an orthopox virus, avipox virus, capripox virus, suipox virus, vaccinia virus, baculovirus, human adenovirus, SV40 or bovine papilloma virus.

45. Use of a mutant *ras* peptide according to claims 1-24 or 27-31 for the manufacture of a medicament for use in a method of preventing the occurrence, inhibiting the growth of or killing tumor cells expressing mutant *ras* p21 protein or peptides comprising:

a. generating mutant *ras* protein or peptide specific cytotoxic T lymphocytes *in vitro* by stimulation of lymphocytes from a source with an effective amount of the mutant *ras* peptide, alone or in combination with one or more

PCT/US97/06470

69
-7471-

cytokines, said amount is effective in generating mutant *ras* protein or peptide specific cytotoxic T lymphocytes; and

b. adoptively transferring the mutant *ras* protein or peptide specific cytotoxic T lymphocytes alone, or with a cytokine into a mammal in an amount sufficient to prevent the occurrence, inhibit the growth or kill the tumor cells.

46. Use of the mutant *ras* peptide according to claim 45 wherein the tumor cells are derived from pancreatic cancer, prostate cancer, lung cancer, colon cancer, melanoma, thyroid cancer, endometrial cancer, oral cancer, laryngeal cancer, seminoma, hepatocellular cancer, bile duct cancer, acute myeloblastic leukemia, basal cell carcinoma, or squamous cell carcinoma.

47. Use of the mutant *ras* peptides according to claims 45 or 46 wherein the method further comprises the administration of a biological response modifier selected from the group consisting of interleukin 2, interleukin 6, interleukin 12, interferon, tumor necrosis factor, GM-CSF and cyclophosphamide.

48. Use of the mutant *ras* peptide according to claim 47, wherein the cytokine is interleukin 2.

49. Use of the mutant *ras* peptide according to claims 45-47 or 48 wherein the method further comprises: step c. administration of a booster amount of a mutant *ras* peptide into the mammal.

50. Use of a mutant *ras* peptide according to claims 1-24 or 27-31 for the manufacture of a medicament for use in a method of preventing the occurrence, inhibiting the growth or killing the tumor cells expressing mutant *ras* p21 protein or peptide in a mammal comprising:

a. generating mutant *ras* p21 protein or peptide specific cytotoxic T lymphocytes *in vivo* by administration of an effective amount of a mutant *ras* peptide alone, or in combination with an adjuvant or liposome formulation, and

WO 97/40156

PCT/US97/06470

70
-751-

b. the mutant *ras* p21 protein or peptide specific cytotoxic T lymphocytes so generated prevent the occurrence, inhibit the growth or kill the tumor cells in the mammal.

51. Use of a mutant *ras* peptide according to claim 50 wherein the adjuvant is selected from the group consisting of RIBI Detox™, QS 21, alum and incomplete Freund's adjuvant.

52. Use of the mutant *ras* peptide according to claims 50 or 51 wherein the tumor cells are derived from pancreatic cancer, prostate cancer, lung cancer, colon cancer, melanoma, thyroid cancer, endometrial cancer, oral cancer, laryngeal cancer, seminoma, hepatocellular cancer, bile duct cancer, acute myeloblastic leukemia, basal cell carcinoma, or squamous cell carcinoma.

53. Use of the mutant *ras* peptides according to claims 51, 51 or 52 wherein the method further comprises the administration of a biological response modifier selected from the group consisting of interleukin 2, interleukin 6, interleukin 12, interferon, tumor necrosis factor, GM-CSF, and cyclophosphamide.

54. Use of a mutant *ras* peptide according to claims 1-24 or 27-31 for the manufacture of a medicament for use in a method of eliciting mutant *ras* p21 protein or peptide specific cytotoxic T lymphocytes comprising:

a. exposing a source containing T lymphocytes to a mutant *ras* peptide, and

b. eliciting mutant *ras* p21 protein or peptide specific cytotoxic T lymphocytes.

55. Use of the mutant *ras* peptide according to claim 54 wherein the source of lymphocytes is peripheral blood, lymph node tissue, tumor tissue or effusions.

56. A mutant *ras* p21 protein or peptide specific cytotoxic T lymphocyte elicited by exposure to a mutant *ras* peptide according to any of claims 1-24 or 27-31.

WO 97/40156

PCT/US97/06470

71
-7641-

57. The mutant *ras* p21 protein or peptide specific cytotoxic lymphocytes according to claim 56 wherein the lymphocytes are cytotoxic to tumor cells expressing mutant K-, H- or N- *ras* protein or peptide.

58. Use of a mutant *ras* peptide according to claims 1-24 or 27-31 for the manufacture of a medicament for use in a method of eliciting mutant *ras* protein or peptide specific cytotoxic T lymphocytes comprising:

a. pulsing antigen presenting cells with a mutant *ras* peptide to form mutant *ras* peptide-pulsed antigen presenting cells; and

b. exposing a source containing T lymphocytes to the mutant *ras* peptide-pulsed antigen presenting cells to elicit the cytotoxic T lymphocytes.

59. Use of the mutant *ras* peptide according to claim 58, wherein the antigen presenting cells are selected from the group consisting of a dendritic cells, B lymphocytes, monocytes and macrophages.

60. Use of a mutant *ras* peptide according to claims 1-24 or 27-31 for the manufacture of a medicament for use in a method of treating cancer in a human comprising: immunization of a human afflicted with a tumor expressing a mutant *ras* p21 protein or peptide with an effective amount of mutant *ras* peptide, said amount is effective in generating a mutant *ras* p21 protein or peptide specific immune response, said immune response is effective in treating the cancer.

61. Use of a mutant *ras* peptide according to claim 60 wherein the cancer is an adenocarcinoma, pancreatic cancer, prostate cancer, colon cancer, lung cancer, endometrial cancer, thyroid cancer, melanoma, oral cancer, laryngeal cancer, seminoma, hepatocellular cancer, bile duct cancer, acute myeloblastic leukemia, basal cell carcinoma, or squamous cell carcinoma.

62. Use of a mutant *ras* peptide according to claims 60 or 61, wherein the immune response is cytotoxicity of the tumor.

63. A mutant *ras* peptide specific cytotoxic T lymphocyte comprising: a T lymphocyte immunoreactive with a mutant *ras* p21 protein or peptide according to claims 1-24 or 27-31 and said T lymphocyte inhibits or kills cells expressing a mutant *ras* p21 protein or peptide wherein the T lymphocyte is MHC class I-

WO 97/40156

PCT/US97/06470

72
-771-

restricted and has a CD8⁺ phenotype, and wherein the MHC Class I is selected from the group consisting of HLA-A2, HLA-A3, HLA-A11, HLA-A68, and HLA-A24.

64. A mutant *ras* peptide specific cytotoxic T lymphocytes according to claim 63 wherein the T lymphocyte is MHC class I HLA-A2 restricted and has a CD8⁺ phenotype.

65. A mutant *ras* peptide specific cytotoxic T lymphocyte according to claim 63 wherein the MHC class I is selected from the group consisting of HLA-A3, HLA-A11, HLA-A68, and HLA-A24.

